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The acid-base condition in vegetation, litter and humus: VIII. Forms of acidity.

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Introduction.

The forms of acidity which we shall discuss here might be grouped according to the following scheme of classification:

A. — Active acidity.
1. Soluble acids (including their salts).
   a. Distillable acids.
   b. Acids decarboxylated by hot 12 % HCl solution (uronic and other acids).
2. Acidoids (colloidal acids).
   a. Hydrolyzable acidoids such as the polyuronides in hemicellulose, pectins and gums.
   b. Nonhydrolyzable acidoids such as those of lignin and ligno-humic acidoids.

B. — Potential acidity.
1. Hydrolytic acidity (by hot 2 % HCl solution).
2. Saponification acidity (by action of 1 n. KOH solution).
3. Oxidation acidity (by autoxidation in 1 n. KOH solution).

Description of materials.

The materials used in this investigation (table 51) represent great variations in composition and properties. The 3 samples (V_1, V_2 & F_0) of leaves (beech, oak, birch, alder, ash and elm) and pine needles are all from the same trees in the Ultuna park. The V_2 samples (green leaves) were collected Sept. 1, 1941 and the F_0 samples (fresh litter) were collected in the same year immediately after their falling. The V_1 samples were collected the following

spring as soon as the leaves appeared fully developed (all in the middle of June with the exception of the ash leaves which, due to the unusually late spring, were not fully developed until after midsummer). The Sphagnum and the lichen are from the Ramna highmoor by Bassholma in southern Sweden and were collected on Aug. 20, 1942. The seaweed Fucus vesiculosus is from Marstrand by Skagerack, and the agar is a commercial product. The Axelstorp podzol humus is from the lower F-layer (about 5—10 cm) in a windborn deposit of beech leaves below a slope in the beech woods by Axelstorp on the Hallandsås ridge in southern Sweden (Mattson and Gustafsson 1934). The Ca-saturated Mölner lowmoor humus is from the same batch as was used in earlier investigations in this series. The lignite was a bricked sample from our geological museum, and the coal is of the type used on the blacksmith's forge. The two samples of »sugar humus» were each prepared from 200 grams of sucrose dissolved in 2 liters 1:1 and 1:3 solutions of concentrated hydrochloric acid and water, by heating the mixtures on the water bath for 40 hours. The yield was 35.6 and 21.5 grams respectively.

The lichen Cladonia rangiferina, the Sphagnum fuscum and the Sphagnum cuspidatum form a hydrologic series, the lichen being collected on the driest part of a hillock, the S. fuscum on its slope, and the S. cuspidatum in a submerged depression all within an area of 2 m^2.

Methods.

The finely ground samples have been titrated with KOH in N. KCl solution (1:20), the pH being determined with the glass electrode. Titration curves have thus been obtained of the original samples, of the electrodialysed residues, of the residues from the hydrolysis with a hot 2 % HCl solution, and of the residues from the hydrolysis with 72 % H_2SO_4 solution. The original samples and the hydrolytic residues have likewise been titrated after an alkaline autoxidation in an atmosphere of oxygen gas. In this way we have been able to obtain a quantitative estimation of the various forms of acidity enumerated in the introduction. A discussion of details is given in connection with each individual experiment. Of the nearly two hundred titration curves we shall here reproduce only a few representative types.

The composition of the original samples.

Tables 51 a and b show the composition of the samples with respect to the fractions which are of interest in connection with the various forms of acidity. The water soluble organic matter was determined on a separate sample by shaking 3 grams with 60 cc

Table 51 a. *Composition of the samples of the basis of 100 grams original organic matter.*

Sample	Species	Soluble in:		Residue after hydrolysis with:		Excess base in:		
		water %	alcohol-benzol 1:1 %	2 % HCl %	72 % H_2SO_4 %	total sample, *B* m. e.	water sol. fraction m. e.	insol. residue (diff.) m. e.
Beech V_1	Fagus silvatica	24.6	16.9	34.4	22.2	71.2	34.6	36.6
» V_2	» »	20.9	18.8	40.3	31.3	88.7	25.5	63.2
» F_0	» »	16.84	17.3	47.5	33.7	106.4	—	—
Alder V_1	Alnus incana	24.3	29.7	25.8	20.5	66.8	32.1	34.7
» V_2	» »	20.6	25.5	31.9	24.5	87.2	31.0	56.2
» F_0	» »	23.6	24.8	30.3	21.3	99.0	36.5	62.5
Oak V_1	Quercus robur	26.5	14.8			58.0	37.7	20.3
» V_2	» »	20.7	17.6			87.8	31.0	56.8
» F_0	» »	21.5	15.9		22.6	105.1	17.1	88.0
Birch V_1	Betula pubescens	24.1				55.1	24.3	30.8
» V_2	» »	23.6				71.0	30.5	40.5
» F_0	» »	19.4	19.9		29.6	115.2	29.0	86.2
Ash V_1	Fraxinus excelsior	18.8				128.6	76.0	52.6
» V_2	» »	26.6				183.9	124.5	59.4
» F_0	» »	27.3	17.1		14.0	216.7	133.5	83.2
Elm V_1	Ulmus glabra	29.5				127.9	54.7	73.2
» V_2	» »	27.7				242.6	132.8	109.8
» F_0	» »	23.1	11.4		14.3	280.7	134.3	146.4
Pine V_1	Pinus silvestris	18.2				19.7	16.0	3.7
» V_2	» »	19.8				23.5	13.3	10.2
» F_0	» »	15.9	27.2		21.8	28.2	8.6	19.6
Sphagnum	Sph. fuscum	3.0	4.3	31.9	11.1	18.3	2.8	15.5
»	» cuspidatum	5.6	4.4	31.5	7.8	18.0	0.4	17.6
Cladonia	Clad. rangiferina	3.4	7.8	16.3	6.3	4.3	1.5	2.8
Fucus	Fucus vesiculosus	23.4	10.7	35.8	24.8	—	36.9	—
Agar	D. A. B. 6		0.7		3.1	31.6		
Wood meal	Pine		6.5		26.6	2.6		
Axelstorp F_3	Beech leave mold		3.8	63.9	57.2	33.0		
Mölner H	Neutral humus		5.4		(64.5)	331.3		
Lignite			9.6		85.8	38.1		
Coal			2.6		99.4	—		

of water in the machine for 18 hours, after which a measured volume of the filtrate was taken for the analysis. The alcohol-benzol (1:1) soluble fraction, which had to be separated before the hydrolysis, was extracted in large Soxhlets holding 25 grams, for two days. The residue was then boiled for 5 hours in a 2 % HCl solution, filtered and washed. This removes the pentosans and the hemicellulose (polyuronides, pectins, gums, etc.) but leaves the cellulose, lignin and most of the nitrogenous compounds (cf. Waksman 1938). The »lignin» was prepared by placing 25 grams of extracted and dried

Table 51 b. *The composition of the samples on basis of 100 grams original organic matter. Acids and acidoids at pH 7* (cf. fig. 51—52).

Sample	pH_0	H	Acidoids:			Acids:				
			total, A	hydrolyzable, A_h	non-hydrolyzable, A_n	a	$A+a$	$\frac{A+a}{B}$	pH_u	N
		m. e.	m. e.	m. e.	m. e.	m. e.	m. e.		calc.	%
Beech V_1	4.20	59	44	25	19	86	130	1.83	2.70	3.11
» V_2	5.20	24	35	10	25	78	113	1.27	1.88	2.36
» F_0	4.77	34	55	25	30	84	139	1.31	1.70	1.00
Alder V_1	4.40	67	66	47	19	69	135	2.02	2.70	3.32
» V_2	5.07	37	71	53	18	53	124	1.42	2.36	3.23
» F_0	4.60	59	85	65	20	71	156	1.58	2.32	2.54
Oak V_1	4.20	116	62			111	173	2.98	3.02	3.66
» V_2	4.96	47	60			74	134	1.53	2.32	3.12
» F_0	4.43	67	70	45	25	101	171	1.63	2.12	1.21
Birch V_1	4.94	40	48			49	97	1.76	2.90	3.42
» V_2	5.15	38	81			30	111	1.56	2.75	2.85
» F_0	4.55	82	82	48	34	115	197	1.71	1.75	0.74
Ash V_1	5.38	37	83			83	166	1.29	2.55	3.46
» V_2	5.40	35	79			139	218	1.19	2.40	2.56
» F_0	5.50	31	95	82	13	151	246	1.14	2.05	1.47
Elm V_1	5.21	55	79			105	184	1.44	2.69	3.88
» V_2	5.65	32	98			177	275	1.13	1.97	2.48
» F_0	5.31	49	136	113	23	194	330	1.18	1.90	0.82
Pine V_1	4.01	51	31			43	74	3.76	3.20	1.89
» V_2	4.45	33	31			26	57	2.43	3.10	1.64
» F_0	3.98	32	34	18	16	27	61	2.16	2.73	0.49
Sph. fuscum	2.82	123	121	102	19	19	140	7.65	2.33	0.59
» cuspid.	3.15	103	88	75	13	34	122	6.78	2.73	1.10
Cladonia	3.79	30	23	16	7	11	34	7.91	3.45	0.79
Fucus		38	181	128	53					1.27
Agar	5.90	4	37			7	44	1.39	2.61	0.36
Wood meal	3.99	16	15			7	22	8.46	3.71	0.06
Axelstorp F_3	3.46	101	135	28	107	3	138	4.18	2.48	1.91
Mölner H	6.32	20	289							3.19
Lignite	3.30	174	191	0	246	27	218	5.72	2.52	0.49
Coal	5.95	1	2			0	2	—		0.08

(at 65° C.) material in a 600 cc beaker together with 150 cc 72 % H_2SO_4. After thorough soaking in an evacuated desiccator the mixture was allowed to stand for 3 hours at 18° C., after which it was diluted to 4 500 cc and boiled for 5 hours, filtered and washed. All residues were finally electrodialysed before the titrations. Samples rich in Ca should be washed with HCl or, still better, boiled with a 2 % HCl solution before the sulphuric acid treatment to prevent the formation of $CaSO_4$, which is difficult to remove. The filtration is greatly facilitated by the hydrolysis of the complex carbohydrates.

The »excess base» was determined by backward titration of the ashes to phenol phthalein.

The free acidity H, the total acidoids A and the acids a were read from the curves as shown in fig. 51, for beech and elm leaves. The $A+a$ curve expresses the sum of acidoids and acids per 100 grams of organic matter and was obtained by titrating the original sample. The A curve, which expresses the quantity of acidoids present in 100 grams original organic matter, was obtained by titrating that fraction of a gram of the electrodialyzed residue which corresponded to one gram of the original sample. If, for example, the loss on electrodialysis was 25 percent, we took only 0.75 gram of the residue for titration. The a curve was obtained by subtracting the A curve from the $A + a$ curve as in our previous work (cf. part VII), but with the difference that instead of using the pH_u (the zero point on the A curve) as the zero point for the a curve, we now used a »calculated pH_u» for the $A + a$ curve as the zero point. This point was obtained by finding the pH on the $A + a$ curve at which an amount of HCl equivalent to the »excess base» had been added (cf. distance B in the figures). We found that, in the case of the materials here studied, the B values, as obtained by the horizontal distance between pH_u and the $A + a$ curve, differed, in many cases, considerably from the »excess base» values. We therefore decided to make the »excess base» values the basis for our calculations.

We are aware of the fact that this method of calculating a is not very dependable. The »excess base» value, B, is not exact, and then we have ignored the basoids by not titrating the A curve over on the acid side. The B value should, theoretically, have been fitted in between this extended A curve and the $A + a$ curve. But we have found in the case of several samples that the electrodialyzed material, at low, and at high pH, buffers as much or even more than the original material; as if the basoid groups and the phenol hydroxyls had been activated (set free) by the electrodialysis. In such cases the distance between the two curves may never equal the B value, not even at the lowest pH.

The hydrolyzable and the nonhydrolyzable acidoids were obtained from the type of curves shown in fig. 52. In this figure the A curve is the same as that in fig. 51, i. e., it expresses the quantity of total acidoids per 100 grams of organic matter in the original sample. The curves marked $L + C$ (= lignin + cellulose) and L (= lignin) show the amount of acidoids in the residues from the 2 percent and the 72 percent acid hydrolysis respectively, from originally 100 grams organic matter. These two curves were obtained from the curves in fig. 53 (which express the acidoid content per 100 gram residue) by multiplying the individual titration values by the percent residue from the hydrolysis with 2 % HCl and 72 % H_2SO_4 respectively (table 51 a, column 5 and 6), and dividing by 100. By

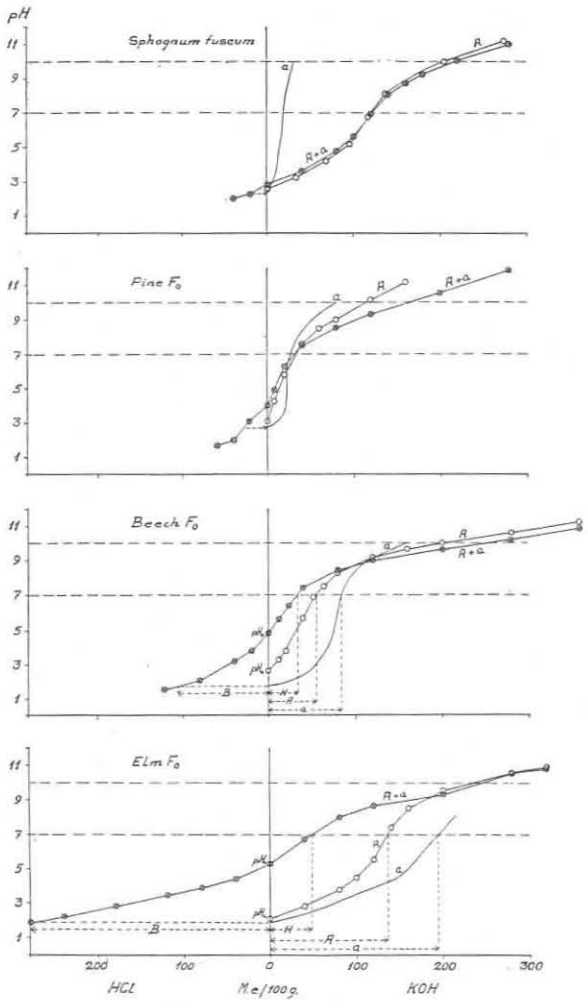


Fig. 51. Titration curves of the original ($A + a$) and of the electrodialyzed (A) samples. The a curve is obtained by subtracting the A curve from the $A + a$ curve. The curves represent quantities present in 100 grams original organic matter (cf. table 51).

subtracting the nonhydrolyzable (»lignin») acidoids A_n (e. g. at pH 7), from the total acidoids we get the hydrolyzable acidoids A_h (cf. beech and elm fig. 52). We can also obtain the entire titration curve of the hydrolyzable acidoids by subtracting the lignin curve from the total acidoid curve A, as has been done for elm (fig. 52).

With this description of methods we are now prepared to discuss the results as given in tables 51 a and b and in fig. 51 and 52.

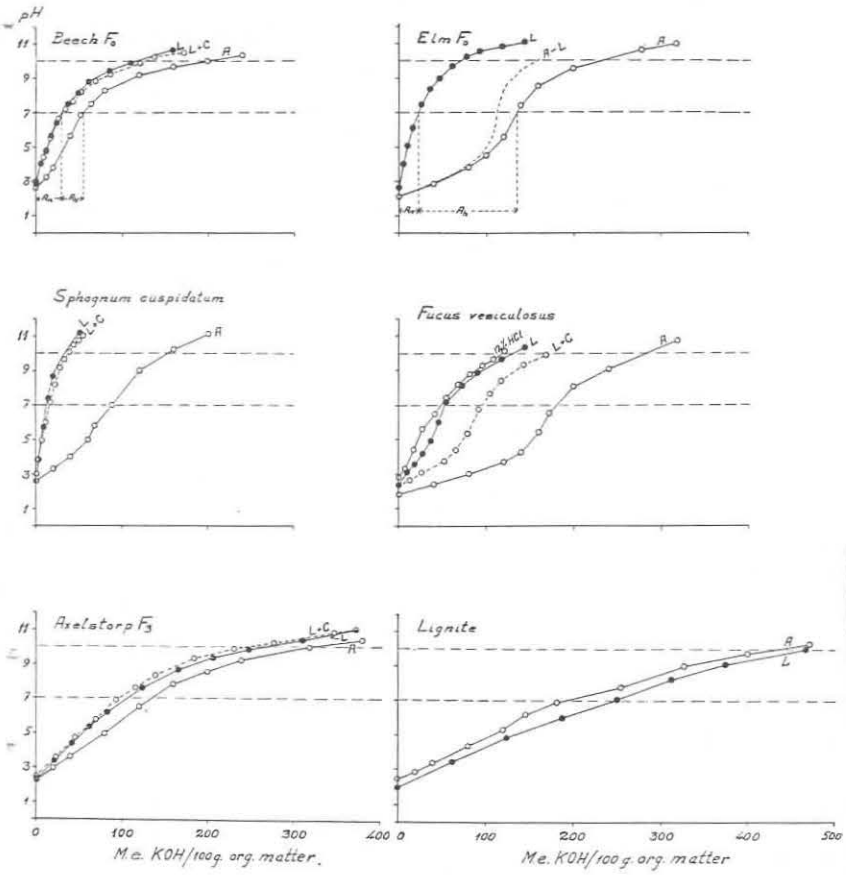


Fig. 52. Titration curves of the electrodialyzed whole samples (*A*, same as in fig. 51), and of the electrodialyzed residues from the 2 percent (L + C) and the 72 percent (L) acid hydrolysis. The curves represent the quantities of the fractions present in 100 grams original organic matter. For elm F_0 the titration curve of the hydrolyzable acidoids has been calculated by subtracting the L curve (= non-hydrolyzable acidoids) from the A curve.

Water soluble organic matter: The extraction with water was done on a separate sample and not, as in the usual course of analysis, after the alcohol-benzol extraction. This yields a greater quantity of organic acids and their salts, but even then the water extraction does not seem to remove more than a certain fraction of the diffusible organic acids removed by electrodialysis (cf. »excess base» in water soluble fraction, which is often much smaller than the *a* values at pH_0 in fig. 51, to be discussed below).

Alcohol-benzol soluble organic matter: This comprises, besides fats, waxes and resins, a certain amount of acidic material which is also soluble in water. This fraction has not otherwise been studied.

The 2 % acid residue: This consists of cellulose, lignin and proteins. The hemicellulose, gums and pectins have been dissolved by the hydrolysis. The sum of the water and alcohol-benzol soluble fractions and of the hydrolytic residue should, when subtracted from 100, give a rough estimation of the amount hydrolyzed. On this basis we find that Sphagnum and lichen have the highest percent hemicellulose whereas beech has the lowest.

The 72 % acid residue: The hydrolysis with strong acid removes the cellulose and leaves a residue which is assumed to consist of lignin and protein, or their derivatives. This nonhydrolyzeable ligno-nitrogenous complex (the »lignin» in our tables and figures) varies between 3.1 percent in the agar and 99.4 percent in the coal. Among the F_o samples it is highest in the beech and lowest in ash and elm. It is very low in the lichen, which is known as a poor humus former. In the Mölner humus it is undoubtedly much higher than the 64.5 percent recorded, because this highly oxidized humus solvates copiously during the washing.

The »excess base»: This has been determined in the water soluble fraction as well as in the total sample. The difference gives the »excess base» which belongs to the insoluble fraction. The »excess base» does not divide itself between the water soluble and insoluble fractions according to the titration values at pH_o on the A and a curves in fig. 51. All the V and F samples show, according to the curves, a greater base combining capacity for the acids than for the acidoids at pH_o, whereas the »excess base» in the water soluble fraction is, according to table 51 a, often considerably lower than the corresponding values for the insoluble residue. This discrepancy is probably due to the insolubility of some of the organic salts, which are, therefore, not extracted by the water but are removed by the electrodialysis.

We note that the »excess base» increases considerably with the maturity of the leaves, being greatest in the F_o samples. It is very low in pine, is still lower in Sphagnum and is extremely low in the lichen. In Fucus it could not be determined due to carbonate incrustations which could only be removed by acid.

The pH_o: Among the V and F_o samples the original pH is, with minor exceptions, lowest in V_1 and highest in V_2. The three high-moor plants have the lowest pH, as would be expected.

The composition of the untreated and autoxidized fractions.

Fig. 53 shows the titration curves of the various fractions, before and after autoxidation, and table 52 gives the acidoid values at pH 7 and 10, the pH_u and the nitrogen content, all based on 100 grams residual organic matter of the electrodialyzed samples. The autoxidation was done in the presence of an excess of calcium hydroxide (20 m. e. per gram organic matter) as explained in parts V and VI of this series. The oxygen consumption is shown in table 53.

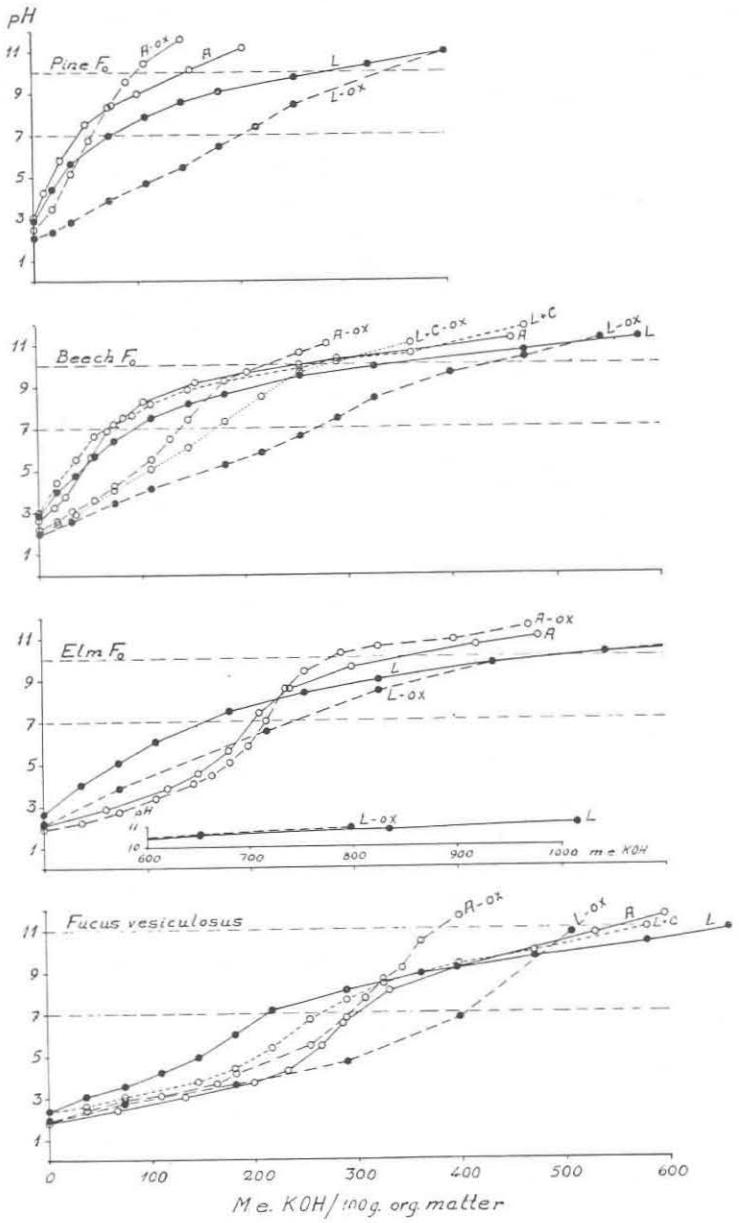
There are *two factors* in the table which bring out some very significant differences and which, on a closer study, reveal some of the most *fundamental relationships* with respect to the different forms of acidity:

The *A* (pH 10) / *A* (pH 7) ratios divide the original samples (table 52 a) into *two groups*, one with low and the other with high ratios. In the group with low ratios we find ash, elm, sphagnum, fucus and the Mölner humus, whereas the other samples fall in the group with high ratios.[1] The removal of the polyuronides by hydrolysis with dilute or with concentrated acid (table 52 c & e) places the ash, elm, sphagnum and fucus in the group having a high *A* (pH 10)/*A* (pH 7) ratio, (the fucus is only partly affected by the weak acid), whereas the Mölner exhibits a low ratio (1.54), even after the most drastic hydrolysis. Further, the removal of the polyuronides causes a general increase in the pH_u values of vegetation and litter, whereas the pH_u of the Mölner humus is decreased by the hydrolysis (cf. also lignite). The less decomposed and less oxidized Axelstorp humus exhibits intermediary changes.

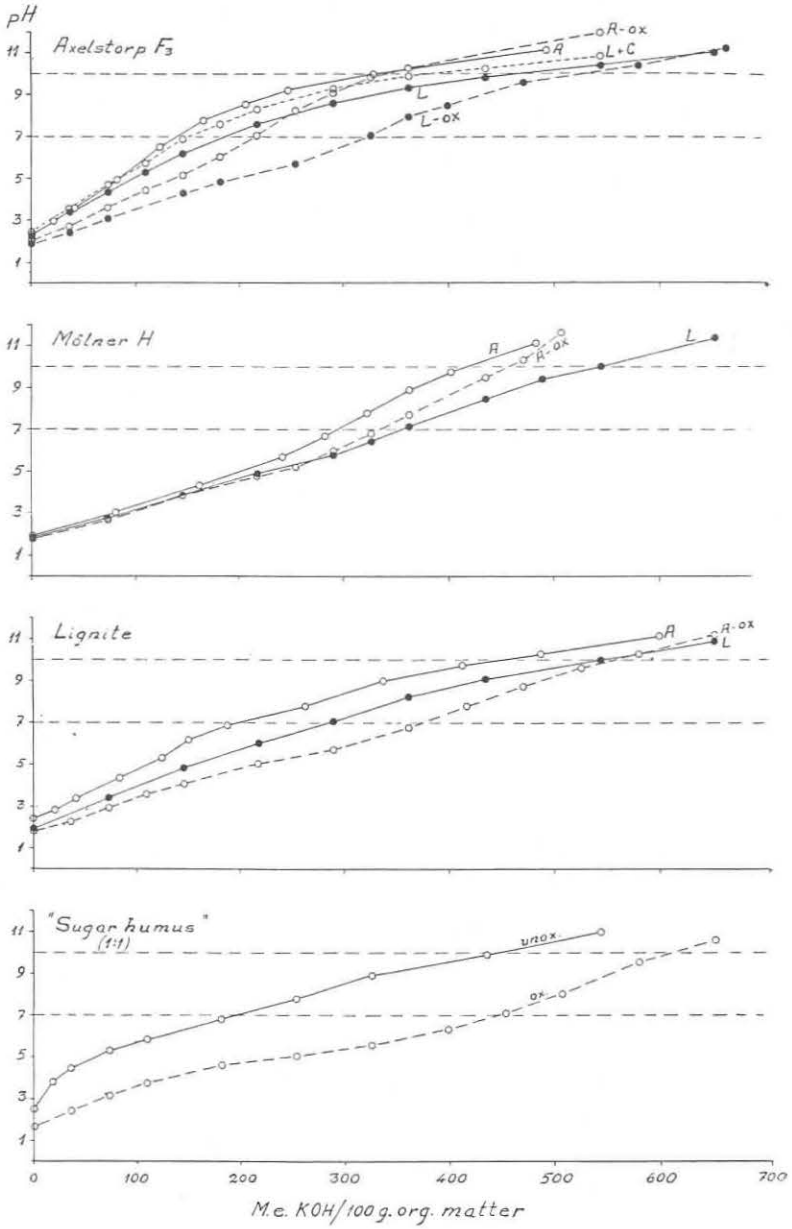
The *autoxidation* leads to a greatly reduced *A* (pH 10) / *A* (pH 7) ratio in all samples with the exception of agar. The latter, which is a sulphuric acid ester of a complex polysaccharide, has undoubtedly undergone an extensive saponification with loss of sulphuric acid during the alkaline autoxidation. Most of the original samples seem to have lost acidity by the alkaline autoxidation, because the increase in the *A* (pH 7) values is often much smaller in table 52 b than in d and f. Three of the original samples, oak F_o, fucus and agar, show even lower *A* (pH 7) values after than before the oxidation. This loss of acidoids is, however, not entirely the result of a saponification. The lignin itself undergoes more or less solvation when, after the autoxidation, the residue is acidified and washed.

How are we now going to explain these group relationships of the different materials? We have seen that ash, elm and sphagnum, and probably also fucus, are rich in hemicellulose (polyuronides)

[1] It should be pointed out that as a result of the great buffering effect at pH 10, the *A* (pH 10) values are not very accurate.



g. 53 a. Titration curves of the various electrodialyzed fractions, all on the sis of 100 grams residual organic matter. A = original acidoids; L + C = sidue from 2 % HCl treatment; L = residue from 72 % H_2SO_4 treatment; ox = autoxidized.


pH
Axelstorp F3
R-ox
A
L+C
L
L-ox
Mölner H
A
A-ox
L
Lignite
A
R-ox
L
"Sugar humus"
(1:1)
unox
ox
0
100
200
300
400
500
600
700
M.e. KOH/100 g. org. matter

Fig, 53 b.

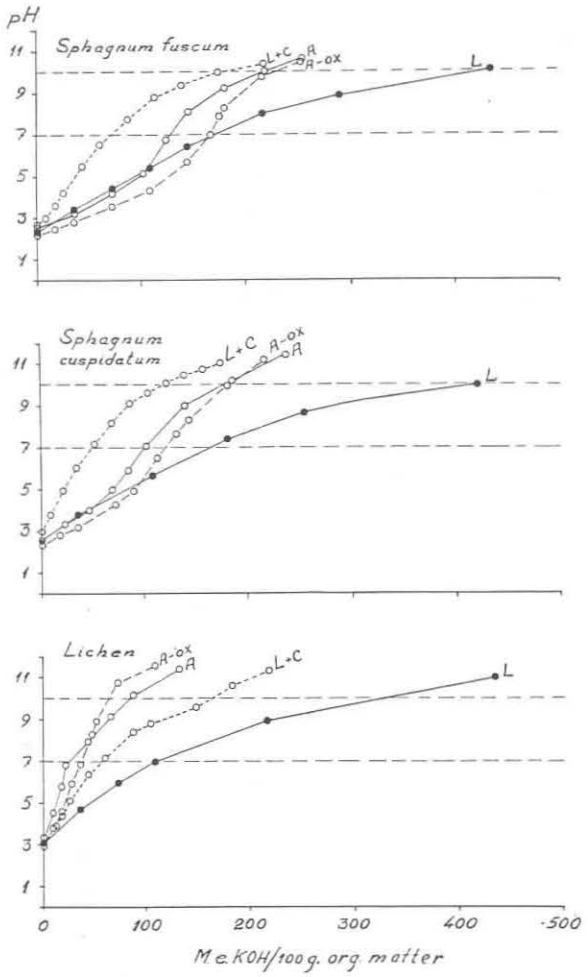


Fig. 53 c.

and poor in lignin, whereas the Mölner humus is very rich in »lignin» and poor in hemicellulose. *The polyuronic acids* are comparatively strong acids. Thus Bonner (1935) found pK values for pectic acid which increased from 2.77 at the beginning to 4.18 at the end of the titration. The form of the curve obtained by Bonner, by direct titration of pectic acid, closely resembles the *A*—*L* curve for elm in fig. 52, which has been plotted by subtracting the »lignin» curve from the total acidoid (*A*) curve in the same figure (cf. also fig. 55). The resulting curve represents the hydrolyzable acidoids in 100

Table 52. *The pH_u and the acidoid and nitrogen content of the untreated and the autoxidized fractions on the basis of 100 grams residual organic matter (cf. fig. 53). All samples electrodialyzed.*

Sample	a. Original:					b. Autoxidized:				
	A (pH 7) m. e.	*A* (pH 10) m. e.	$\frac{A\ (pH\ 10)}{A\ (pH\ 7)}$	pH_u	N %	*A* (pH 7) m. e.	*A* (pH 10) m. e.	$\frac{A\ (pH\ 10)}{A\ (pH\ 7)}$	pH_u	N %
Beech V_1	73	211	2.89	3.19	4.15	139	187	1.35	2.42	4.50
» V_2	49	188	3.84	3.18	3.07	135	211	1.56	2.37	3.70
» F_0	69	255	3.70	2.60	1.05	137	219	1.60	2.14	1.36
Alder V_1	91	293	3.22	3.00	4.28	185	257	1.39	2.41	5.27
» V_2	91	228	2.51	2.85	3.88	163	221	1.36	2.39	4.76
» F_0	119	284	2.39	2.61	2.94	150	209	1.39	2.29	3.87
Oak V_1	99	347	3.51	3.45	5.42	104	151	1.45	2.81	5.46
» V_2	78	295	3.78	3.18	4.20	94	147	1.56	2.94	4.68
» F_0	98	342	3.49	2.62	1.43	90	139	1.54	2.37	1.61
Birch V_1	75	222	2.96	3.39	4.79	129	184	1.43	2.66	5.08
» V_2	106	255	2.41	2.98	3.61	113	169	1.50	2.63	4.44
» F_0	107	326	3.05	2.53	.87	125	169	1.35	2.12	1.07
Ash V_1	111	209	1.88	2.70	4.22	142	177	1.25	2.40	4.51
» V_2	105	187	1.78	2.70	4.35	145	183	1.26	2.30	4.55
» F_0	138	237	1.72	2.30	1.94	156	195	1.25	2.10	2.35
Elm V_1	129	244	1.89	2.73	4.56	155	211	1.36	2.35	5.27
» V_2	159	257	1.62	2.43	3.30	181	249	1.38	2.17	3.83
» F_0	203	350	1.72	2.10	.96	217	281	1 29	1.90	1.08
Pine V_1	43	148	3.44	3.50	2.21	81	130	1.60	2.56	2.17
» V_2	39	145	3.72	3.40	1.87	58	105	1.81	2.67	1.82
» F_0	43	148	3.44	3.09	.46	57	99	1.74	2.50	.49
Sph. fuscum	129	220	1.71	2.55	.60	168	230	1.37	2.17	.51
» cuspid.	103	178	1.73	2.60	.82	121	182	1.50	2.35	.77
Cladonia	26	85	3.27	3.32	.48	37	64	1.73	2.90	.57
Fucus	300	470	1.57	1.80	1.78	294	357	1.21	1.90	2.26
Agar	62	137	2.21	2.16	.26	36	83	2.31	2 38	.24
Wood meal	15	63	4.20	3.35	.05	25	47	1.88	2.79	.06
Axelstorp F_3	139	329	2.37	2.31	2.13	216	339	1.57	2.06	2.38
Mölner H	293	416	1.42	1.93	3.74	334	457	1.37	1.80	4.06
Lignite	198	450	2.27	2.40	.86	374	557	1.49	1.79	.54
Coal	2	7	3.50	4.51	—	—	—	—	—	—
»Sugar humus» (1:1)	196	446	2.28	2.51	—	447	611	1.37	1.65	—
»Sugar humus» (1:3)	177	483	2.73	2.46	—	441	640	1.45	1.58	—

grams of elm leaves. The other samples yield similar curves. We note the striking contrast (opposite convexity) between this form of curve and that of the original, unoxidized »lignin» curves. *The »lignins»*, even that of the elm leaves which have a high base status are weaker acidoids, with relatively high pH_u. The high acidoid content and low pH_u of litter, rich in bases, is evidently not due to any particularly high state of oxidation of the lignin, as we

Table 52 (continued).

Sample	c. Residue from 2 % HCl treatment:					d. The same as c, autoxidized:				
	A (pH 7) m. e.	A (pH 10) m. e.	A (pH 10) / A (pH 7)	pH_u	N %	A (pH 7) m. e.	A (pH 10) m. e.	A (pH 10) / A (pH 7)	pH_u	N %
Beech V_1	67	244	3.64	4.22	2.37	134	216	1.61	2.49	2.41
» V_2	65	294	4.52	3.91	2.01	181	305	1.69	2.28	2.25
» F_0	66	267	4.05	2.99	.71	172	281	1.63	2.03	.85
Alder V_1	82	266	3.24	3.45	3.88					
» V_2	100	280	2.80	2.77	3.45					
» F_0	79	235	2.97	3.29	2.63					
Sph. fuscum	71	177	2.49	2.70	.53					
» cuspid.	50	119	2.38	3.00	.64					
Cladonia	57	165	2.89	2.88	.90					
Fucus	266	478	1.80	2.35	1.26					
Axelstorp F_3	150	385	2.57	2.47	2.22					
	e. Residue from 72 % H_2SO_4 treatment (= »lignin»):					f. »Lignin», autoxidized:				
Beech V_1	88	295	3.35	3.69	4.09	241	359	1.49	2.29	4.19
» V_2	78	264	3.38	3.68	3.16	247	364	1.47	2.23	3.58
» F_0	93	347	3.73	2.87	1.28	272	442	1.63	1.99	1.47
Alder V_1	94	331	3.52	4.30	5.18	299	445	1.49	2.40	5.60
» V_2	73	288	3.95	4.31	5.15	246	363	1.48	2.35	5.75
» F_0	98	339	3.46	3.91	4.19	225	368	1.64	2.49	—
Oak F_0	112	392	3.50	3.22	1.85	225	380	1.69	2.16	2.04
Birch F_0	116	370	3.19	2.73	1.13	258	394	1.53	1.95	1.21
Ash F_0	92	284	3.09	3.50	2.97	143	252	1.76	2.76	3.14
Elm F_0	159	500	3.14	2.63	2.01	245	496	2.02	2.15	—
Pine F_0	73	284	3.89	2.90	.83	202	342	1.69	2.09	.82
Sph. fuscum	173	430	2.49	2.35	1.57					
» cuspid.	165	420	2.54	2.59	2.53					
Cladonia	110	330	3.30	3.04	2.22					
Fucus	213	530	2.49	2.36	1.70	407	486	1.19	1.90	—
Wood meal	23	89	3.87	3.29	.16	85	167	1.96	2.61	.22
Axelstorp F_3	187	468	2.50	2.25	1.83	324	526	1.62	1.89	1.95
Mölner H	353	544	1.54	1.81	3.36					
Lignite	285	544	1.91	1.90	.68					
Coal	2	5	2.50	5.12	—					

have believed, but to a high content of hydrolyzable acidoids. (The »functional» lignins of the leaves have, however, a much higher acidoid content than the »structural» lignin of the wood meal. Note also the relatively low acidoid content of the untreated and autoxidized ash »lignin», which might be ascribed to the woody stalks of these leaves.)

The autoxidized »lignins» yield curves resembling that of the Mölner humus. This humus, which is of the chernozem or »Grauhuminsäuren» type, is so highly »humified» and autoxidized that a further,

Table 53. *The oxygen consumption during 5 days autoxidation in the presence of* $Ca(OH)_2$.

Sample	Liters O_2 consumed on the basis of 100 g:						
	original organic matter:				fractionated organic matter:		
	Whole sample	Water soluble fraction	2 % HCl residue	72 % H_2SO_4 residue	Water soluble fraction	2 % HCl residue	72 % H_2SO_4 residue
Beech V_1	5.36	2.09	2.88	2.39	10.20	8.39	10.80
» V_2	5.95	1.80	4.02	3.76	15.40	9.98	12.01
» F_0	6.79	1.85	4.79	4.86	15.08	10.08	14.45
Alder V_1	8.73	—	—	2.36	—	—	11.51
» V_2	6.05	—	—	2.24	—	—	9.13
» F_0	5.84	—	—	1.71	—	—	8.02
Oak F_0	4.55	—	—	1.86	—	—	8.20
Birch F_0	4.82	—	—	3.05	—	—	10.29
Ash F_0	3.21	—	—	.60	—	—	4.27
Elm F_0	2.89	—	—	.90	—	—	6.30
Pine F_0	2.73	—	—	1.68	—	—	7.70
Fucus	5.98	—	—	3.25	—	—	13.10
Wood meal	.69	—	—	.84	—	—	3.16
Axelstorp F_3	5.52	—	—	5.23	—	—	9.14

The following values represent the whole sample as in column 2 above.

Sample	L./100 g.	Sample	L./100 g.	Sample	L./100 g.
Oak V_1	5.03	Pine V_1	2.93	Coal	.10
» V_2	3.45	» V_2	2.43	»Sugar humus» (1: 1)	11.44
Birch V_1	4.16	Sph. fuscum	2.63	» » (1: 3)	13.16
» V_2	3.55	» cuspid.	1.87		
Ash V_1	2.64	Cladonia	1.34		
» V_2	2.54	Agar	.83		
Elm V_1	2.83	Mölner	2.37		
» V_2	2.41	Lignite	7.20		

artificial autoxidation leads to an extensive solvation when the acidified residue is washed, and later when electrodialyzed. The acidoid content, the A (pH 10)/A (pH 7) ratio and the pH_u are, therefore, only slightly affected by the autoxidation of the Mölner. The similarity between the curves of the autoxidized »lignin» and of the Mölner humus strengthens our conviction that the humus complex represents autoxidized (and ammoniated) lignin (cf. general discussion).

We are perhaps now prepared to answer the above questions concerning the group relationships. The low A(pH 10) / A(pH 7)

ratios in the original ash, elm, sphagnum and fucus on the one hand, and in the Mölner humus on the other, are due to very different constituents. The former contain a large proportion of strong, polyuronic acidoids, which, on hydrolysis, change to soluble acids, leaving a residue of the less strongly acidic lignin. Upon autoxidation the lignin assumes strong acidoid properties, in place of the weak, phenolic, which disappear. The Mölner humus, on the other hand, consists of highly autoxidized lignin acidoids, which are not subject to hydrolysis. The property of the humus remains therefore nearly constant. *A titration curve of litter or vegetation acidoids can be resolved into two very different curves, each representing one of two major types of acidoids* (cf. elm fig. 52). The form of the original (compound) curve of a V or F sample (and of the incompletely transformed and autoxidized »Braunhuminsäuren») will vary with the proportions of these two types of acidoids (cf. beech and elm fig. 52). *The curve of a humus like the Mölner cannot be thus resolved.*

The form of the titration curves is also modified by the proportion of *basic nitrogen*. The acidoid content, as expressed by the curves, is smaller than the true content by an amount equivalent to the inner salt formation. The true content of strong acidoids in the V samples, which are rich in nitrogen, must therefore be considerably greater than the apparent content indicated by the curves. For the same reason the pH_u values are always high in the V samples.

The titration curves of some desaminated samples are shown in a special paper in this volume.

It is significant that in humus there is a different relationship between the pH_u and the nitrogen content. In humus we have always found a high nitrogen content to go with a high acidoid content and a low pH_u (cf. part IV and VI). This relationship might be looked upon as a natural result of a progressive autoxidation in conjunction with an oxidative ammonia fixation.

It is interesting to note that the »*sugar humus*» shows a very high acidoid content, and that the autoxidation more than doubles the A(pH 7) values. The autoxidation, which changed the color from black to reddish brown, has, as in the natural samples, caused a considerable decrease in the weak acidoids groups (phenol hydroxyl). There is no marked differences between the two samples.

Since the »sugar humus» was not included in our paper on »ammonia fixation and humus nitrogen» it might be of interest to add here that the autoxidation of the two samples in the presence of 12 % NH_3 led to a fixation of 8.29 percent nitrogen in the 1:1 sample, and 10.13 percent in the 1:3 sample of »sugar humus». The corresponding amounts of displaceable NH_4-nitrogen was 4.01 and 4.35 percent. This makes a total nitrogen content of 12.30 and 14.48 percent, respectively. We might consider this ammonia fixation as an

evidence of a phenolic structure, resulting from a dehydration of the sugar, for example

$$C_6H_{12}O_6 - 3\,H_2O = C_6H_3(OH)_3\,.$$

Table 53 shows the amounts of *oxygen consumed* by the autoxidation. In the case of beech, which includes all the fractions, we note that the sum of the volumes of oxygen consumed by the water soluble fraction and the hydrolytic residue (either from the 2 percent or from the 72 percent acid) is roughly equal to the volume consumed by 100 grams of the original organic matter. This indicates that the carbohydrats fraction consumes little or no oxygen.

The autoxidation is very high in the water soluble material (due probably to tannins and related materials), in the »lignins» of beech, alder (V_1) and fucus, and in »sugar humus». Note that the wood »lignin» is the least active form. We note also that the already highly oxidized Mölner consumes much less oxygen then the lignite and the Axelstorp humus. The 72 percent acid residue of the latter consumes nearly as much oxygen as the original sample.

Hydrolytic, saponification, and oxidation acidities.

For this study we selected materials from widely different sources (Table 54). The oat hay is from a special cylinder experiment intended for a study of the acid-base condition in relation to different fertilizer treatments. The hay is from 1943, and was cut on June 18. The clover hay is from the sample cut on July 9 1942, and described in part VII of this series. The beech and ash leaves, the Axelstorp and Mölner humus, and the beech leaf lignin are all from the same electrodialyzed samples as described in the preceding tables. The tannin and the fructose were both of the highest purity chemicals. The aqueous extracts are from our 1943 collection of leaves.

The titrations were carried out in large test tubes, 25 mm in diameter and 300 mm long. The test tubes were provided with a perforated rubber stopper fitted with a glass tube. This tube was sealed at the lower end, but was instead provided with a side opening about 1 cm above the end. Properly lubricated with glycerin, this tube can be pushed down into the test tube until a communication is established, or it can be pulled back into the stopper, thus closing the test tube. This arrangement works very smoothly.

Four portions of each sample, equal to 2 grams organic matter (tannin = 0.5 g.), were placed in a series of four test tubes: 1, for the determination of the original acidity; 2, for the hydrolytic acidity; 3, for the saponification acidity, and 4, for the oxidation acidity. Tube 1 received 75 cc 1 n. KCl solution. Tube 2 received 25 cc 1.085 n. HCl (= 4 % HCl) and 25 cc 2 n. KCl solution, was heated 5 hours in a water bath under a reflux condenser (a 60 cm

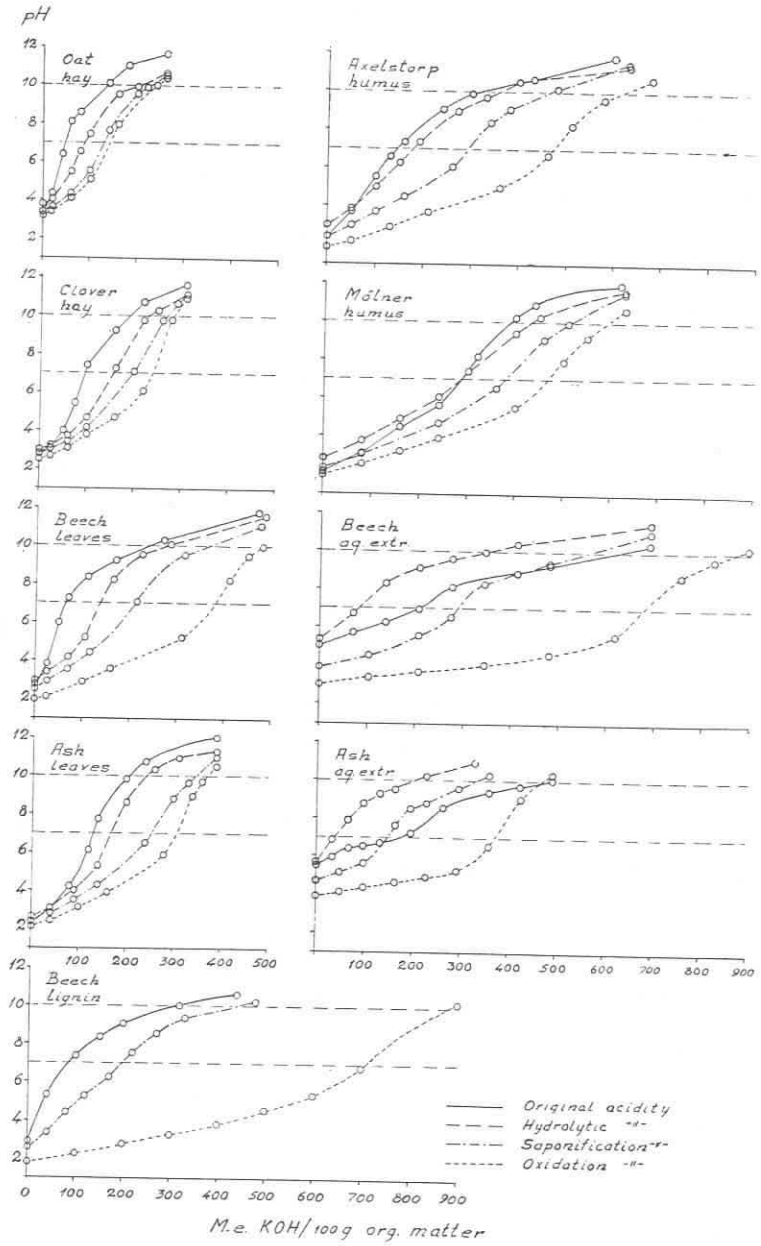


Fig. 54 a. Titration curves of (1) the original, electrodialyzed samples; (2) after boiling with 2 percent HCl; (3) after anaerobic treatment with 1.085 n. KOH and (4) after autoxidation in the presence of 1.085 n. KOH.

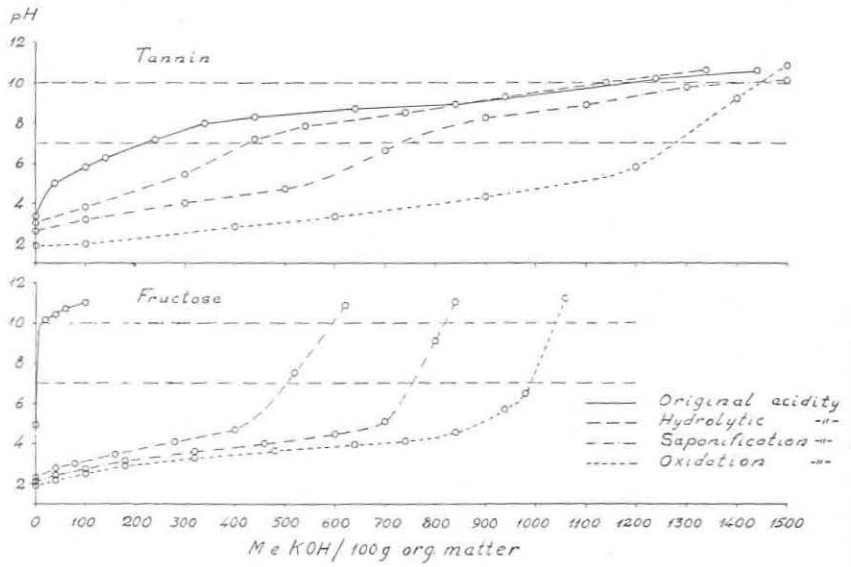


Fig. 54 b.

long glass tube), after which 25 cc 1.085 n. KOH were added. Tube 3 received 25 cc 1.085 n. KOH, was immediately evacuated and kept 5 days at 37° C. after which 25 cc 1.085 n. HCl and 25 cc 2 n. KCl were added. Tube 4 received 25 cc 1.085 n. KOH, was immediately evacuated and placed in the constant temperature room, was filled with oxygen and shaken in the machine for 5 days, during which time the tube was twice refilled with oxygen. After the autoxidation the tube received 25 cc 1.085 n. HCl and 25 cc 2 n. KCl solution.

The liquid extracts were prepared by shaking, for 18 hours, a portion of the leaves, corresponding to 25 grams organic matter, with 250 cc water, and filtering through a G. 3 glass filter funnel. Volumes of 25 cc, corresponding to 2.5 grams original organic matter, and equal to 0.291 grams water soluble organic matter in the case of beech leaves, and 0.611 grams in the case of ash leaves, were taken for the titrations. We then proceeded as in the case of the solid samples, with the difference that, since we had no room for the KCl solution, we added the calculated amount of dry KCl. Another difference was that the KOH solution, being diluted by an equal volume of extract, was here only one half as concentrated during the saponification and autoxidation.

The variously treated suspensions and solutions, which were approximately 1 n. with respect to KCl, were now titrated with standard KOH solution. After each addition of the base the tubes

were rapidly evacuated, shaken in the machine from 4 P. M. until 9 A. M. on the third day when the pH was determined by the glass electrode in a small volume of the supernatant liquid, which was then returned to the test tube. More base was now added and the procedure repeated.

We consider this continuous method of titration very convenient and about as accurate as it would be to carry out the complete pretreatments for each individual titration, on a very much greater number of weighed samples. The only additional errors the method introduces is the change in the KCl concentration, which may be as much as 10 percent reduced, and the loss of a drop or two of the supernatant liquid at each pH determination.

Most of the carbonic acid is removed during the first evacuation of the acidified systems.

Table 54 is taken from the titration curves in fig. 54 which are plotted to only one half of the scale of the preceding curves.

We note by the curves that the autoxidation produces the greatest amounts of acidity and yields the strongest acids. In tannin the increase is 1053 m. e./100 g. and in fructose 989 m. e./100 g. In beech leaf lignin the increase is 620, in water soluble beech leaf organic matter 486, in Axelstorp F_3 (beech mold) 324, and in the nondialyzable beech leaf residue it is 314 m. e./100 organic matter.

The high potential acidity of beech leaves is significant, for it accounts for the fact that the soil under a stand of beech so often is podzolizied. It is obvious that a litter possessing as low a base content and as high a potential acidity as the beech leaves, must constitute a severe tax on the base status of the soil. The oxidation acidity of the beech leaves is considerably greater than that of the ash leaves which possess more than twice the amount of base and which are much more readily decomposed (»mineralized») (cf. part I).

The low oxidation acidity of the oat hay reflects the low activity of cereal straw lignin.

It is interesting to note that a large part, often the largest part (cf. fructose), of the oxidation acidity is developed by the mere anaerobic treatment with alkali. The increase in acidity due to »saponification» is, in other words, in some cases greater than the increase due to autoxidation, if we measure the latter by the difference between them. How much of the »saponification» acidity actually is due to a saponification of esters, and how much of it is developed through an intramolecular oxidation-reduction we do not know. In the case of fructose the reaction must be wholly of the latter type (cf. extensive literature on the oxidation of sugars, some of which was discussed in part V of this series).

The original acidity curve of the aqueous extract of the ash leaves shows a peculiar deviation from the course of the other

Table 54. *»Hydrolytic» acidity, »saponification» acidity and oxidation acidity of various forms of organic matter* (cf. fig. 54).

Sample	Acidity per 100 g. organic matter by titration: to pH 7: Orig. m. e.	Hydr. m. e.	Sap. m. e.	Ox. m. e.	to pH 10: Orig. m. e.	Hydr. m. e.	Sap. m. e.	Ox. m. e.	Oxygen consumed per 100 g. Liters
Oat hay, electrodial.	46	91	128	141	138	212	232	240	1.23
Red clover, »	94	154	199	238	190	234	268	284	1.63
Beech F_0, »	66	139	213	380	240	276	356	480	7.48
Ash F_0, »	132	170	255	307	208	246	343	370	2.60
Axelstorp F_3 »	146	178	296	470	320	360	463	616	6.50
Mölner H, »	287	284	378	466	389	434	515	591	4.45
Beech F_0 lignin, »	88	—	200	708	320	—	444	878	15.30
Tannin	220	424	727	1 273	1 188	1 140	1 412	1 445	25.60
Fructose	0	503	754	989	12	595	818	1 039	9.78
Beech F_0 aq. extr.	208	81	291	694	616	348	558	872	13.40
Ash F_0 » »	172	38	149	372	490	210	332	473	6.88

Sample	Acidity pH 10 / Acidity pH 7: Orig. m. e.	Hydr. m. e.	Sap. m. e.	Ox. m. e.	Acidity increase: at pH 7: Hydr. m. e.	Sap. m. e.	Ox.[1] m. e.	at pH 10: Hydr. m. e.	Sap. m. e.	Ox.[1] m. e.
Oat hay, electrodial.	3.00	2.33	1.81	1.70	45	82	13	74	94	8
Red clover, »	2.02	1.52	1.35	1.19	60	105	39	44	78	16
Beech F_0, »	3.64	1.99	1.67	1.26	73	147	167	36	116	124
Ash F_0, »	1.58	1.45	1.35	1.21	38	123	52	38	135	27
Axelstorp F_3, »	2.19	2.02	1.56	1.31	32	150	174	40	143	153
Mölner H, »	1.36	1.53	1.36	1.27	–3	91	88	45	126	76
Beech F_0 lignin, »	3.64	—	2.22	1.24	—	112	508	—	124	434
Tannin	5.40	2.69	1.94	1.14	204	507	546	–48	224	33
Fructose	∞	1.18	1.08	1.05	503	754	235	583	806	221
Beech F_0 aq. extr.	2.96	4.30	1.92	1.26	–127	83	403	–268	–58	314
Ash F_0 » »	2.85	5.53	2.23	1.27	–134	–23	223	–280	–158	141

curves in that it intersects the »saponification» acidity curve below pH 7. At the time the work was done we did not suspect that this could be the result of an anaerobic fermentation. The system was 1 n. with respect to KCl, and toluol had been added to the aqueous extracts. Other experiments, just now completed, indicate that such fermentation is possible in nutritious, aqueous extracts, not otherwise sterilized (as when HCl or KOH is added). Since an excess of toluol affects the rubber, such systems must be sterilized by heat.

[1] Oxidation acidity minus saponification acidity.

The »hydrolytic» acidity curves show that the treatment with hot 2 percent HCl solution has increased the acidity considerably in the two samples of hay, in the F_0 samples of beech and ash, in tannin and in fructose. In the case of the Axelstorp and Mölner humus the hydrolytic acidity is so small that the effect of a decarboxylation (cf. below) dominates at the lower end of the curves. Note that this effect is greatest in the Mölner. In the two aqueous extracts the acid treatment has caused a great decrease in acidity. There is apparently very little hydrolyzable organic matter in solution, but a large amount of easely decarboxylated acids such as uronic and other hydroxy acids.

The hydrolytic acidity is here seen to be the net result of two opposing reactions; 1. the formation of acids by hydrolysis, 2. the destruction of acids by decarboxylation. Different concentration of HCl and different time of treatment would probably result in different amounts of hydrolytic acidity. The optimum conditions for the production of acidity will no doubt vary for different types of organic matter.

In fructose there is formed everything from simple organic acids (formic, levulinic) to the complex »sugar humus» acidoids, all depending upon the strenght of acid and time and temperature of treatment. The reaction with the fructose is, of course, here again an intramolecular oxidation-reduction reaction and not a mere hydrolysis.

What has been said above with respect to the position of the curves, is shown with figures in table 54.

We note here the large amounts of oxygen consumed by all the samples of beech, whether it be the original sample, the lignin or the aqueous extract.

There is only a poor proportionallity between the amounts of oxygen consumed and the oxidation acidities, but then the carbon dioxide is not included in the latter.

The acidity ratios (pH 10/pH 7) have all been reduced by the treatment with acid or alkali, except in the case of the hydrolytic acidity of the Mölner and of the two aqueous extracts. It is evidently only relatively strong acids and acidoids which are formed by hydrolysis, by saponification and by autoxidation. It is therefore no wonder that the humus acidoids become stronger with progressive humification, and stronger the higher the base status.

Decarboxylation and uronic acid content.

When the uronic acids are boiled with strong mineral acids each molecule loses one molecule of carbon dioxide and is thereby converted into the corresponding pentose sugar

$$CHO(CHOH)_4CO_2H = CHO(CHOH)_3CH_2OH + CO_2,$$

which then breaks down further to give furfural and water

$$C_5H_{10}O_5 = C_5H_4O_2 + 3\,H_2O.$$

The method for determining uronic acids, originally proposed by Lefèvre and Tollens and later modified by Dickson, Otterson and Link (1930), consists in measuring the amount of CO_2 evolved when the sample is boiled with 12 % HCl solution.

The method has been applied to peat and soils by Shorey and Martin (1930) and by Waksman and Reuszer (1932) and others. While their work leaves no doubt as to the presence of uronic acids in peat and soil, it provides more than one interpretation of the results, from a quantitative point of view.

Shorey and Martin found the furfural yield to be far below the theoretical in all the mineral soils (12.5 to 35.0 percent of calculated), and that prolonged boiling to insure complete decarboxylation in no case increased the yield; this despite the fact that the soils must have contained some free pentosans. The authors account for this on the assumption that the reactivity of furfural leads to the formation of reversion products or furan derivatives.

Waksman and Reuszer discuss this relationship between furfural and carbon dioxide and suggest that the low yield of furfural »may also be due to too high a figure obtained by calculating the uronic acid from the CO_2 liberated by hydrolysis». Citing Norman and Martin (1930), who found pure pectin to yield more CO_2 than could be accounted for by the uronic acids present, they continue: »If this is true of pure pectin, it may certainly hold true of peat and especially of mineral soils, which contain a number of other complexes capable of liberating CO_2 on continued boiling with 12 per cent HCl, even if the carbonates are removed with dilute acid.»

Waksman and Reuszer proceed, however, to base their calculations of the uronic acid anhydride on the amount of CO_2 evolved by the soil and find, among other results, that the organic matter of the B_1 horizon of a pine forest soil consists of 32.39 percent uronic acid anhydride and only 2.27 percent total pentosan. The same organic matter is calculated to contain 36.20 percent crude protein but only 32.30 percent lignin derivatives. The cellulose is reported to be 1.10 percent and the hemicellulose 9.09 percent. The relative increase in uronic acids is ascribed to a greater stability and to a synthesis by microorganisms. Bacterial slimes and gums are rich in uronic acids, it is pointed out.

We find it hard to believe that the once solvated and in the B horizon precipitated humus acidoids can contain so much uronic acid, not to mention the »protein». The fact that we have found very little hydrolyzable acidoids and practically no hydrolytic acidity in humus makes the presence of large amounts of uronic

acid complexes very improbable, as does also the small amount of pentosan reported by Waksman and Reuszer.

Believing that a determination of the amount of CO_2 split off from our original and autoxidized samples by boiling with 12 percent HCl might contribute to a solution of the problem, we procured the apparatus described by Dickson, Otterson and Link and carried out the analyses shown in table 55.

The samples were first boiled with sufficient HCl to combine with all the bases ($pH < 2.0$) until CO_2 no longer was given off. The suspension was then made 12 percent with respect to HCl, and air from two soda-lime towers was drawn through the apparatus for 20 minutes. The contents of the flask were then boiled for 4 hours in a paraffin bath at 142—145° C.

The CO_2 was determined by titrating the excess of barium hydroxide.

We note in the table that some of the acids and acidoids formed by autoxidation yield CO_2 upon boiling with 12 percent HCl. The increase is greatest in the case of beech lignin, a complex which, least of all, can be suspected to form uronic acids by autoxidation.

We note also that the 2 percent HCl-treated fucus which we, from the form of its titration curve, suspected to be incompletely hydrolyzed, does yield large amount of CO_2, some of which undoubtedly comes from residual polyuronides.

The two samples of sphagnum yield large amounts of CO_2, whereas the lichen has a low yield, all in agreement with their titration curves.

The aqueous extract of ash leaves yield about twice as much CO_2 as that of beech leaves, but then the former contained about twice as much organic matter (24.44 %) as the latter (11.64 %). The increase in CO_2 due to autoxidation of the beech leaf extract is relatively large.

It is interesting to note that the autoxidized »sugar humus» yields considerable CO_2 by boiling with 12 percent HCl. Since this product is itself formed by boiling sugar with a still stronger HCl solution, we may consider the original »sugar humus» stable in 12 percent HCl. Although the autoxidized product had been electrodialysed after first being washed with acid, we found that a considerable quantity of CO_2 was given off during the preliminary boiling. We therefore repeated the distillation without the preliminary boiling and got a yield of 104.5 m. e. CO_2 per 100 grams. This indicates that some of the CO_2 is loosely bound. The same conclusion was reached by Waksman and Reuszer who determined the CO_2 split off, both with and without the 1 percent HCl pretreatment recommended by Shorey and Martin.

Many of the hydroxy acids lose CO_2 easely. Gortner (1938) points out that malic acid, when exposed to sunlight, loses 2 mole-

cules of CO_2 and forms ethyl alcohol. Our experiment has shown that some of the carboxyl acids formed by the oxidation of lignin are also unstable.

Whatever the nature of the compounds which split off CO_2 when humus is boiled with 12 percent HCl, one thing seems certain: not all the CO_2 comes from uronic acids.

A determination of the potential acidities by a quadruple titration of the organic matter in the various horizons of a podzol profile should be illuminating.

Table 55. *The evolution of CO_2 by distillation with 12 % HCl. (M. e. × 0.022 × 4 would give percent uronic anhydride.)*

Sample	CO_2 m. e./100 g.	Sample	CO_2 m. e./100 g.
First ten same as in table 54[1]:		Sphagnum fusc. untreated ...	199.8
		Sphagnum cuspid. untreated .	147.8
Beech F_0 original	119.8	Cladonia rangiferina untreated	51.1
do autoxidized ...	157.7	»Sugar humus» (1 : 1) autoxidized.....	83.7
Ash F_0 original	221.6	Beech F_0 1943 untreated	116.7
do autoxidized ...	250.0	do autoxidized	175.2
Axelstorp original	129.6	do aqueous extr.[3] untreated	11.6
do autoxidized ...	167.1	do aqueous extr.[3] autoxidized	19.7
Mölner original	85.9	Ash F_0 1943 aqueous extr. untreated[3]	22.0
do autoxidized ...	111.4		
Beech lignin original	26.0		
do autoxidized ...	115.0		
Fucus vesic. untreated...	226.4		
do 2 % HCl treated	146.5[2]		

Distillable acids.

The distillable acids have been determined in the untreated and autoxidized ash and beech F_0 samples of our 1943 collection. The distillation was done with steam on 2 grams of organic matter suspended in 1 n. sulphuric acid. A volume of 1200 cc was distilled over, boiled free from CO_2 under a reflux, and titrated to phenolphthalein. Results:

Beech F_0	untreated	60.7	m. e./100 g.
do	autoxidized	115.4	» »
Ash F_0	untreated	35.0	» »
do	autoxidized	82.9	» »

[1] These ten samples were the originally electrodialysed samples used in, and left over from the titration experiment table 54.

[2] = 52.5 m. e. per 100 g. original organic matter

[3] From 100 g. original organic matter.

Since the suspension had to be acidified, we decided to use such an excess of acid as would be likely to bring about a complete hydrolysis, otherwise the results would not have been comparable. The last 400 cc of the distillate contained about 10 percent of the total acids distilled over from the untreated samples, and less than half that much in the case of the autoxidized samples.

We note that the hydrolyzed leaves yield large amounts of distillable acids and that the beech leaves, which originally contain less acids and yield less CO_2 upon boiling with 12 percent HCl than the ash leaves, contain nearly twice as much distillable acids.

The autoxidation leads to the formation of large amounts of distillable acids, the increase being somewhat greater in the beech leaves.

General conclusions.

In fig. 55 we see three distinct types of titration curves. Curves 1, 2 and I closely resemble Bonner's pectic acid curve (inserted), and are the curves of relatively strong acids. Curves 1 and 2 represent the titration of the aqueous extracts from 100 grams of beech and ash leaves (F_0, 1942)[1] respectively. The extracts were titrated to pH 10 with KOH and to pH 2 with HCl, both in the presence of 1 n. KCl. Curve I represents the hydrolyzable acidoids in elm leaves (cf. fig. 52) and has been obtained by subtracting the nonhydrolyzable acidoids from the total acidoids as explained.

Curve II represents the nonhydrolyzable acidoids in 100 grams beach leaf (F_0) »lignin». It is the curve of a weak acidoid, indicating a low carboxyl content but a very high content of phenol hydroxyl groups.

Curve III represents the same material as curve II after 5 days of autoxidation in the presence of an excess of $Ca(OH)_2$. The curve shows a very high content of strong acid groups (carboxyl acids or oxy-quinones or both) and a relatively low content of phenol hydroxyl, which apparently have been destroyed or strengthened by the autoxidation.

Curve IV is the curve of the Mölner humus, that is, it is the curve of a highly humified and autoxidized humus, a »Dauerhumus» or »Grauhuminsäure».

The striking similarity between the titration curve of autoxidized »lignin» and that of the Mölner humus is very significant, for it offers a strong support of the theory that the specifically humic acidoids are formed by an autoxidation of the lignin. The fact that the Mölner

[1] These extracts were from the same leaves as described in table 43 A, part VI which, by mistake, were stated to be from the 1941 collection. The percentage water soluble organic matter was 9.05 and 31.60 in the beech and ash leaves respectively.

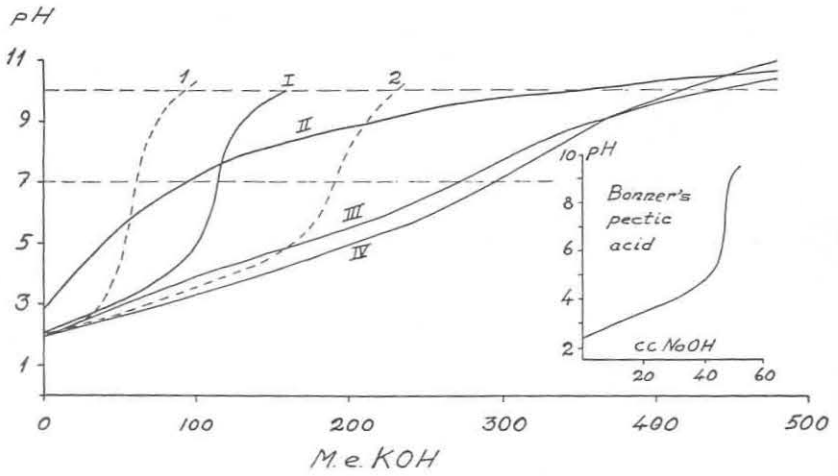


Fig. 55. Titration curves representing: 1 and 2. Aqueous extracts from 100 grams beech and ash leaves (F_0) respectively;

I. Hydrolyzable acidoids in 100 grams of elm leaves (cf. fig. 52);
II. 100 grams of beech F_0 »lignin».
III. 100 grams autoxidized beech F_0 »lignin».
IV. 100 grams Mölner humus. (Fig. 53.)

humus is almost exclusively composed of nonhydrolyzable acidoids lends further weight to this theory.

Summary.

The study includes various forms of vegetation (green leaves, mosses, lichen, algae) litter and humus. Artificial »sugar humus», fructose and tannic acid have also been included.

A distinction is made between active and potential acidity.

The active acidity is divided into soluble acids, hydrolyzable and nonhydrolyzable acidoids. The soluble acids have been fractionated into distillable acids and acids which, on boiling with 12 percent HCl, split off CO_2. The hydrolyzable acidoids include the polyuronides of the hemicellulose fraction (pectins, gums, mucilages), whereas the nonhydrolyzable are represented by the »lignin» acidoids.

By subtracting the »lignin» titration curve from the total acidoid curve we get a third curve which represents the hydrolyzable acidoids, and which has a form very different from the »lignin» curve. The hydrolyzable acidoids consist almost exclusively of relatively strong acid groups, whereas the nonhydrolyzable acidoids contain a preponderance of weak phenol hydroxyl groups.

Different forms of vegetation and litter may differ greatly with respect to the relative proportion of the two forms of acidoids.

The specifically humic acidoids are of the nonhydrolyzable type. Their titration curves resemble closely the titration curves of autoxidized »lignin».

Humus and lignite were found to contain little or no hydrolyzable acidoids. The CO_2 split off on boiling the humus with 12 percent HCl is not believed to be due to the presence of uronic acids (except in small amounts). Autoxidized »lignin» gives off large amounts of CO_2 when boiled with 12 percent HCl.

The potential acidity is divided into hydrolytic acidity, saponification acidity and oxidation acidity. A method for determining the potential acidities is described.

The potential acidity is very high in beech leaves as compared to ash leaves. Since the base content of the former is less than half that of the latter, their different relationship to soil formation is pointed out.

Humus yields little or no hydrolytic acidity. The saponification and oxidation acidities were smaller in a neutral than in an acid humus.

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